Etoposide-induced blood-brain barrier disruption

Effect of drug compared with that of solvents

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The intracarotid infusion of the anti-neoplastic compound, etoposide, has been shown to exert a dose-dependent effect on blood-brain barrier (BBB) permeability. Etoposide, however, is formulated in a complex solvent solution containing alcohol, Tween 80, polyethylene glycol 300, and citric acid. To investigate the contribution of the solvent solution to BBB disruption, the authors studied Sprague-Dawley rats after the internal carotid artery infusion of the solvent solution with and without the addition of etoposide. Experiments were performed at four doses of drug and/or solvent. Disruption of the BBB was evaluated qualitatively by the appearance of the systemically administered dye, Evans blue, in the cerebral hemispheres and quantitatively by the ratio of gamma counts of the technetium-labeled chelate of diethylenetriaminepentaacetic acid ($^{99m}$Tc-DTPA) in the ipsilateral:contralateral hemisphere. Significant barrier opening was obtained in all four groups of animals infused with solvent plus etoposide. In the corresponding groups of rats infused with the solvent solution alone, BBB disruption was markedly lower. Only in the group infused with the largest dose of solvent was the hemispheric ratio of $^{99m}$Tc-DTPA significantly different from saline-infused animals. Each of the groups with solvent plus etoposide had $^{99m}$Tc-DTPA ratios significantly different from the control group. Intracarotid infusion and subsequent BBB disruption were well tolerated by the animals receiving either solvent alone or solvent and etoposide. Disruption of the BBB secondary to the intracarotid infusion of etoposide is primarily caused by the drug itself and not by the solvent solution.

KEY WORDS · blood-brain barrier · etoposide · rat model · intracarotid infusion · drug delivery · chemotherapy

The role of the blood-brain barrier (BBB) in response of malignant brain tumors to chemotherapy remains controversial. There is evidence, however, that the BBB is completely intact in small central nervous system (CNS) neoplasms and at least partially intact in many larger tumors. This evidence forms a basis for the hypothesis that the relative impermeability of the BBB to most chemotherapeutic drugs is an important factor in the treatment of these malignancies.

One experimental approach currently under investigation is the search for methods that can increase BBB permeability and, therefore, allow for delivery of therapeutic concentrations of chemical agents. We have recently presented evidence that the intracarotid infusion of the clinical formulation of the anti-neoplastic compound, etoposide (4 demethyl-epipodophyllotoxin-$\beta$-D-ethylidine glucoside), can disrupt the BBB in a rat model system. Because of its relative water insolubility, etoposide is formulated and supplied in a solution containing 400 mg Tween 80, 3.25 gm polyethylene glycol 300, 10 mg anhydrous citric acid, 150 mg benzyl alcohol, and absolute alcohol amounting to 5 ml for each 100 mg of etoposide. This formulation of etoposide was found to increase BBB permeability in the rat model system, and therefore it was unclear whether this effect was due to the drug and/or the solvent. Disruption of the BBB secondary to the intracarotid administration of alcohol alone was first described in 1945 by Broman and Lindberg-Broman and further characterized in 1962 by Lee. Potential effects of Tween 80, polyethylene glycol 300, or citric acid on BBB permeability have to our knowledge never been investigated. Potential biological activity of the solvent solution alone was manifest in initial toxicological studies of etoposide, where the dose of the solvent solution...
were infused with the clinical formulation of etoposide. One group received 5 ml of normal saline. Four groups received a total volume of 5 ml. The remaining four groups received 22.5 mg/kg (1.125 ml/kg), diluted in normal saline to (drug and solvent) at doses of 3.0 mg/kg (0.15 ml/kg), 7.5 mg/kg (0.375 ml/kg), 15.0 mg/kg (0.75 ml/kg), or 22.5 mg/kg (1.125 ml/kg), diluted in normal saline to a total volume of 5 ml. During the procedure. After the carotid infusion, 0.5 ml of a 2% solution of Evans blue dye and 150 μCi of \( ^{99m} \text{Tc-DTPA} \) in 0.1 ml of normal saline was injected into the femoral vein catheter. Rats were decapitated 2 hours after the end of the intracarotid infusion, since this elapsed time ensured minimal intravascular residue of \( ^{99m} \text{Tc-DTPA} \). This compound has a steep plasma decay curve secondary to rapid urinary excretion. After removal of the brains, staining of the cerebral hemispheres by Evans blue dye was evaluated by direct visual inspection and was graded as follows: Grade 0, no staining; Grade 1+, just noticeable staining; Grade 2+, moderate staining; Grade 3+, dark staining. The brains were sagittally hemisected and the dura mater, cerebellum, brain stem, pituitary gland, and pinnal gland were removed. The hemispheres were weighed and radioisotope was counted in a gamma counter to an error of less than 1%.

Materials and Methods

**Experimental Protocol**

Experiments were performed on female Sprague-Dawley rats, each weighing 300 to 350 gm, that had free access to pellet food and tap water until the time of surgery. All rats were anesthetized with xylazine (Rompun), 12 mg/kg intramuscularly, and ketamine (Ketalar), 30 mg/kg intraperitoneally. Atropine, 0.15 mg/kg, was given subcutaneously. A polyethylene catheter was placed into the left femoral vein. Another polyethylene catheter was inserted in a retrograde manner into the left external carotid artery to the bifurcation of the common carotid artery, as described by Rapoport.

Nine groups of eight rats each were studied. Each animal received a constant infusion* of 5 ml total volume over 25 minutes into the left internal carotid artery via the catheter in the external carotid artery. One group received 5 ml of normal saline. Four groups were infused with the clinical formulation of etoposide (drug and solvent) at doses of 3.0 mg/kg (0.15 ml/kg), 7.5 mg/kg (0.375 ml/kg), 15.0 mg/kg (0.75 ml/kg), or 22.5 mg/kg (1.125 ml/kg), diluted in normal saline to a total volume of 5 ml. The remaining four groups received the etoposide solvent solution† alone in volumes identical to the above groups and also diluted in normal saline to the above volumes. During the intracarotid infusions, the pterygopatine branch of the internal carotid artery, which does not supply the brain, was temporarily occluded with an aneurysm clip. Normal blood flow carried the infusate up the left internal carotid artery to the brain. Neither the common carotid nor the internal carotid artery was ever occluded during the procedure. After the carotid infusion, 0.5 ml of a 2% solution of Evans blue dye and 150 μCi of \( ^{99m} \text{Tc-DTPA} \) in 0.1 ml of normal saline was injected into the femoral vein catheter. Rats were decapitated 2 hours after the end of the intracarotid infusion, since this elapsed time ensured minimal intravascular residue of \( ^{99m} \text{Tc-DTPA} \). This compound has a steep plasma decay curve secondary to rapid urinary excretion. After removal of the brains, staining of the cerebral hemispheres by Evans blue dye was evaluated by direct visual inspection and was graded as follows: Grade 0, no staining; Grade 1+, just noticeable staining; Grade 2+, moderate staining; Grade 3+, dark staining. The brains were sagittally hemisected and the dura mater, cerebellum, brain stem, pituitary gland, and pinnal gland were removed. The hemispheres were weighed and radioisotope was counted in a gamma counter to an error of less than 1%.

**Statistical Methods**

Radioisotopic counts of the cerebral hemispheres were analyzed as the ratio of counts per unit weight in the left to right hemisphere. There was a wide range among the variances of the ratios in the groups. Even after transformation of the ratios to the natural logarithms, there was still a statistically significant difference between the variances. Therefore, the distributions of the ratios among the groups were compared using non-parametric methods (Kruskal-Wallis analysis of variance on the ranks). Comparisons of the control group with each of the other groups were done using multiple comparisons based on the Kruskal-Wallis rank sums with an overall level \( \alpha = 0.05 \).

**Results**

Table 1 presents the effects of the intracarotid infusions on Evans blue staining of the left cerebral hemispheres. None of the brains in the saline control group was stained. All brains in the group receiving 0.15 ml/kg (3.0 mg/kg) etoposide plus solvent were stained, but none of the corresponding solvent alone group had evidence of Evans blue staining. As the etoposide dose increased, there were progressively higher grades of staining. At the highest etoposide dose, 1.125 ml/kg (22.5 mg/kg), one rat had Grade 1+ staining, five had Grade 2+, and two had Grade 3+. In the animals receiving solvent alone, there was also evidence of increased effect with higher doses, but not to the extent seen in etoposide-infused animals. With the largest solvent dose, 1.125 ml/kg, seven of eight rats had Evans blue dye visible in the left hemisphere, but three were graded as 1+, four as 2+, and none as 3+.

Evans blue staining of the contralateral (right) hemi-

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* Syringe infusion pump, Model 351, manufactured by Sage Instruments, Division of Orion Research, Inc., 840 Memorial Drive, Cambridge, Massachusetts.

† Solvent solution without etoposide supplied by Bristol Laboratories, Syracuse, New York, the manufacturer of etoposide.

‡ Searle Model 1185 gamma counter manufactured by Tracor Analytic, Inc., 1842 Brummel Drive, Elk Grove Village, Illinois.
TABLE 1

Intensity of left cerebral hemispheric staining with Evans blue dye after internal carotid artery infusions

<table>
<thead>
<tr>
<th>Infusate</th>
<th>Evans Blue Staining Grade†</th>
<th>0</th>
<th>1+</th>
<th>2+</th>
<th>3+</th>
</tr>
</thead>
<tbody>
<tr>
<td>normal saline</td>
<td></td>
<td>8</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>etoposide &amp; solvent</td>
<td></td>
<td>0</td>
<td>6</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>0.150 ml/kg (3.0 mg/kg)</td>
<td></td>
<td>0</td>
<td>5</td>
<td>3</td>
<td>0</td>
</tr>
<tr>
<td>0.375 ml/kg (7.5 mg/kg)</td>
<td></td>
<td>0</td>
<td>1</td>
<td>6</td>
<td>1</td>
</tr>
<tr>
<td>0.750 ml/kg (15.0 mg/kg)</td>
<td></td>
<td>0</td>
<td>1</td>
<td>5</td>
<td>2</td>
</tr>
<tr>
<td>1.125 ml/kg (22.5 mg/kg)</td>
<td></td>
<td>8</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

* All doses were diluted in normal saline to a total volume of 5.0 ml and were infused over 25 minutes. There were eight rats in each treatment group.
† Staining grades: 0 = none; 1+ = just noticeable; 2+ = moderate; 3+ = dark.

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TABLE 2

Medians and ranges of hemispheric ratios of 99mTc-DTPA after internal carotid artery infusions

<table>
<thead>
<tr>
<th>Infusate</th>
<th>Median Ratio</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>normal saline</td>
<td>1.05</td>
<td>0.96–1.41</td>
</tr>
<tr>
<td>etoposide &amp; solvent</td>
<td>2.49</td>
<td>1.55–2.98</td>
</tr>
<tr>
<td>0.150 ml/kg (3.0 mg/kg)</td>
<td>2.99</td>
<td>2.19–3.53</td>
</tr>
<tr>
<td>0.375 ml/kg (7.5 mg/kg)</td>
<td>3.17</td>
<td>2.72–4.42</td>
</tr>
<tr>
<td>0.750 ml/kg (15.0 mg/kg)</td>
<td>3.37</td>
<td>2.43–5.47</td>
</tr>
<tr>
<td>1.125 ml/kg (22.5 mg/kg)</td>
<td>3.37</td>
<td>2.43–5.47</td>
</tr>
<tr>
<td>solvent alone</td>
<td>1.30</td>
<td>1.12–1.68</td>
</tr>
<tr>
<td>0.150 ml/kg</td>
<td>1.53</td>
<td>1.19–1.98</td>
</tr>
<tr>
<td>0.375 ml/kg</td>
<td>2.08</td>
<td>1.26–3.23</td>
</tr>
<tr>
<td>0.750 ml/kg</td>
<td>2.38</td>
<td>1.47–3.95</td>
</tr>
</tbody>
</table>

* All doses were diluted in normal saline to a total volume of 5.0 ml and were infused over 25 minutes. There were eight rats in each treatment group.

Fig. 1. Relationship of the hemispheric ratios of gamma counts of 99mTc-DTPA to the volume of solvent alone or solvent and etoposide in the intracarotid infusion. Ratios of gamma counts (cpm/gm) in the left hemisphere to those in the right hemisphere are expressed as means with associated 95% confidence limits.

sphere was very common. All rats with Grades 2+ or 3+ staining in the left (infused) hemisphere had evidence of Evans blue in the right hemisphere also, usually in the frontal lobe. All of the animals without Evans blue in the left hemisphere had no dye visible in the right hemisphere. Eight of the 21 rats with Grade 1+ staining on the side of the infusion showed some staining in the right hemisphere also.

Figure 1 depicts the mean ratios of the concentration of 99mTc-DTPA between the left and right cerebral hemispheres. In the saline-treated control group, the mean ratio was 1.11:1. For the animals infused with solvent alone, the mean ratios increase to 1.30, 1.53, 2.08, and 2.38:1 with increasing volumes of solvent. For the groups of rats infused with etoposide and solvent, the mean ratios increase to 2.33, 2.91, 3.43, and 3.62:1 for comparable volumes of solvent containing etoposide.

The median ratios and the minimum and maximum ratios for each of the groups are presented in Table 2. The ranked distribution of the ratios among the nine experimental groups are significantly different (Kruskal-Wallis analysis of variance, χ² = 53.79, p = 0.0001). Multiple pairwise comparisons of the groups at an overall level α = 0.05 reveal that, whereas all the etoposide-infused groups are significantly different from the saline-treated control group, only the group receiving the highest volume of solvent (1.125 ml/kg) is statistically different from the saline group.

The intracarotid infusion was well tolerated by the rats in each of the nine groups. All animals had at least begun to move around spontaneously by the time of sacrifice. No focal neurological deficits were detected, although the rats with Grade 3+ Evans blue staining tended to remain sluggish until the time of sacrifice.

Discussion

The re-emergence of intracarotid administration of chemotherapeutic drugs, including etoposide, in the treatment of CNS neoplasms calls for careful study of potentially different effects of these drugs when given by this method rather than by the more conventional intravenous route. One such possible effect is on BBB permeability. This is especially important in light of the potential for increase in both chemotherapeutic efficacy and toxicity after experimental manipulation of the BBB. In our initial screening of the effect of the intracarotid infusion of various anti-neoplastic
Blood-brain barrier disruption by etoposide

agents on the BBB, etoposide was found to have the capability of significantly altering BBB permeability.24 However, because of the complex solvent solution utilized in the clinical formulation of this drug,14 and past evidence that alcohol alone can affect BBB permeability,15 it was unclear whether it was the etoposide or the solvent solution that was affecting the BBB.

The present findings indicate that, while the solvent solution alone is capable of altering BBB permeability, the major effect is due to the drug itself. It appears that at low to moderate doses, etoposide produces consistent disruption, whereas solvent alone has negligible if any effect on BBB permeability. Since the lower doses of 3.0 mg/kg and 7.5 mg/kg approximate the dose range used in intravenous administration of etoposide in man,13 BBB modification may be achievable in man with intracarotid use of this drug. It is unknown whether the doses of solvent alone that are necessary for producing significant barrier disruption in the rat would be tolerable in man.

The finding of Evans blue dye in the hemisphere contralateral to the side of infusion is common to many techniques of BBB disruption.4,10 This is frequently the result of a common anterior cerebral artery supplying both frontal lobes.30 Therefore the ratio of isotopic counts is, especially at the higher grades of disruption, an underestimate of the true increase in 99mTc-DTPA in the disrupted areas.

The sluggishness observed in rats with Grade 3+ Evans blue staining of the left hemisphere was the only sign of toxicity observed in this study. Since none of the rats infused with solvent alone had Grade 3+ Evans blue staining, and all of the rats treated with the highest dose of solvent (1.125 ml/kg) recovered uneventfully from the carotid infusion, this toxicity is most likely related to the degree of disruption and not directly to the amount of infused solvent. Toxicity associated with Grade 3+ barrier opening has been found with other techniques of BBB disruption.20,25 Potential long-term toxicity from intracarotid etoposide is under investigation.

The mechanism of etoposide- or solvent-induced BBB disruption is unknown. Initial light microscopic studies of etoposide-infused rats have shown no histopathological changes either in the cerebral vasculature or in the rest of the brain parenchyma. Electron microscopic examination of these tissues is currently in progress. Known potential causes of BBB disruption, such as hypertension, hyperosmolar perfusion of the brain, seizures, and abnormal PaCO2, have not been found in etoposide-treated rats.

Other chemotherapeutic agents merit study of potential drug and solvent effects on BBB permeability. This is especially true for compounds such as carmustine (BCNU) and teniposide (VM-26), both of which have shown activity in CNS neoplasms, have been given by the intracarotid route, and include alcohol in their solvent solutions.5,7,27,29

References
21. Rapoport SI: Osmotic opening of the blood-brain barrier,

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